STREAM SALAMANDER MONITORING: NORTHEAST REFUGES AND PARKS

Northeast Amphibian Research and Monitoring Initiative

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INTRODUCTION

Stream salamanders are receiving more attention as ecological indicators (Roth et al., 1999; Ohio EPA, 2001). Stream salamanders in the family Plethodontidae often replace fish as the top vertebrate predators in headwater stream ecosystems. Headwater habitats are the small swales, seeps (where ground water oozes slowly to the surface, usually forming a pool), creeks, and first order streams that form the origins of larger rivers. Stream salamanders are promising indicators of environmental stressors in small streams due to their longevity, relatively stable populations, small home ranges, abundance, and ubiquity (Rocco and Brooks, 2000; Welsh and Ollivier, 1998). Studies have found reduced salamander species richness or abundance in streams with higher impervious surface area in the basin (Boward et al., 1999), increased urbanization (Orser and Shure, 1972) and acid mine drainage (Middlekoop et al., 1999; Rocco and Brooks, 2000), and with nearby road construction (Welsh and Ollivier, 1998) and logging (Bury and Corn, 1988; Corn and Bury, 1989). To monitor changes in populations of stream salamanders in relation to environmental variables, efficient and effective standardized sampling techniques that detect and accurately characterize presence and abundance of all species and age classes are essential.

Stream salamanders are most active at night, avoiding predation by diurnal vertebrate predators. During the day, they hide under or in different types of microhabitat cover including rocks, logs, leaves, moss, bark, burrows and overhanging banks. Stream salamanders are often difficult to survey because they can escape into crevices and interstices among rocks along the stream and stream bank (Pauley and Little, 1998). Survey techniques do not sample all species with equal efficiency (Fellers, 1997) and almost certainly differ in their ability to detect larvae and adults. Estimating attributes of larval populations is important because larvae may be more sensitive than adults to environmental stressors (e.g., stream acidification, Kucken et al., 1994; Middlekoop et al., 1999; Rocco and Brooks, 2000). The presence of larval salamanders indicates the population is reproducing and resident in the stream on an annual basis. Comparisons of stream amphibian sampling methods have been conducted to identify effective monitoring techniques (Fellers and Freel, 1995; Jung et al., 2000; Mitchell, 1998a,b, 1999; Pauley and Little, 1998; Welsh, 1987). From these studies, quadrat and transect methods appear to be efficient in capturing larvae and adults and promising for long-term monitoring.

This project seeks to answer the following questions: 1) What is the status of stream salamander populations on protected lands in the Northeast? and 2) Are they effective indicators of ecological condition in small streams undergoing some form of degradation or disturbance? Examples of disturbance could include logging or clear cutting in the adjacent riparian zone, the presence of horse or cow pastures along the stream, a recent fire, storm water runoff, urbanization, point sources (e.g., maintenance yard drainage), acid mine drainage, and nearby roads or road construction. The objectives of this project are to: (1) conduct transect and quadrat sampling for stream salamanders, (2) determine salamander detection rates and population estimates along transects, (3) obtain data from a range of degraded and non-degraded sites, and (4) establish a long-term stream salamander monitoring program on Federal lands within the Northeast. Only first- and second-order streams (those likely draining less than 1,000-acre catchments) will be surveyed. Predominant land use will be used to designate streams as developed (>25% urban or 50% agriculture) or undeveloped (>50% forested). Using GIS, upstream catchment land use will be calculated and used in analyses. Classification of sites as to degree of degradation will be based on the surveyor's assessment of disturbance or degradation, land use, and physical habitat.

Biologists with the U.S. Fish & Wildlife Service, National Park Service, and U.S. Geological Survey will participate in the stream salamander surveys. In 2004, our goal is to survey a minimum of 8 stream sections at each of 5 Refuges and 3 National Parks (Table 1) using quadrat and transect methods. Typically this will consist of picking 4 streams and conducting two transects and two quadrats per stream. A stream section consists of one transect and one quadrat (a transect-quadrat pair). We will estimate stream salamander populations at all transects using removal sampling based on at least three removal passes (Bruce, 1995; Rexstad and Burnham, 1991; Salvidio, 1998).

Table 1. National Parks and National Wildlife Refuges (NWR) where stream salamander surveys will be conducted in 2004.

Stream Salamander Survey Sites 2004	*No. Degraded Stream Sections*	*No. Undegraded Stream Sections*
Rock Creek Park	7	7
Shenandoah National Park	9	9
Acadia National Park	4	4
Canaan Valley NWR	4	4
Wallkill River NWR	4	4
Lake Umbagog NWR	4	4
Patuxent NWR	4	4
Moosehorn NWR	4	4
TOTAL	40	40

MATERIALS AND METHODS

Stream and Transect Site Selection

A stream is defined as a "surface watercourse having a channel with well defined bed and banks, either natural or artificial, which confines and conducts continuous or periodical flowing water" (Ohio EPA, 2001). You can locate first and second order stream sections (see Figure 1) using either 7.5 minute series USGS topographic maps, aerial photos, or USDA Natural Resources Conservation Service county soil maps. Small headwater streams are not often visible at the USGS 1:24,000 mapping scale, but can be found on foot from the streams you are able to find.

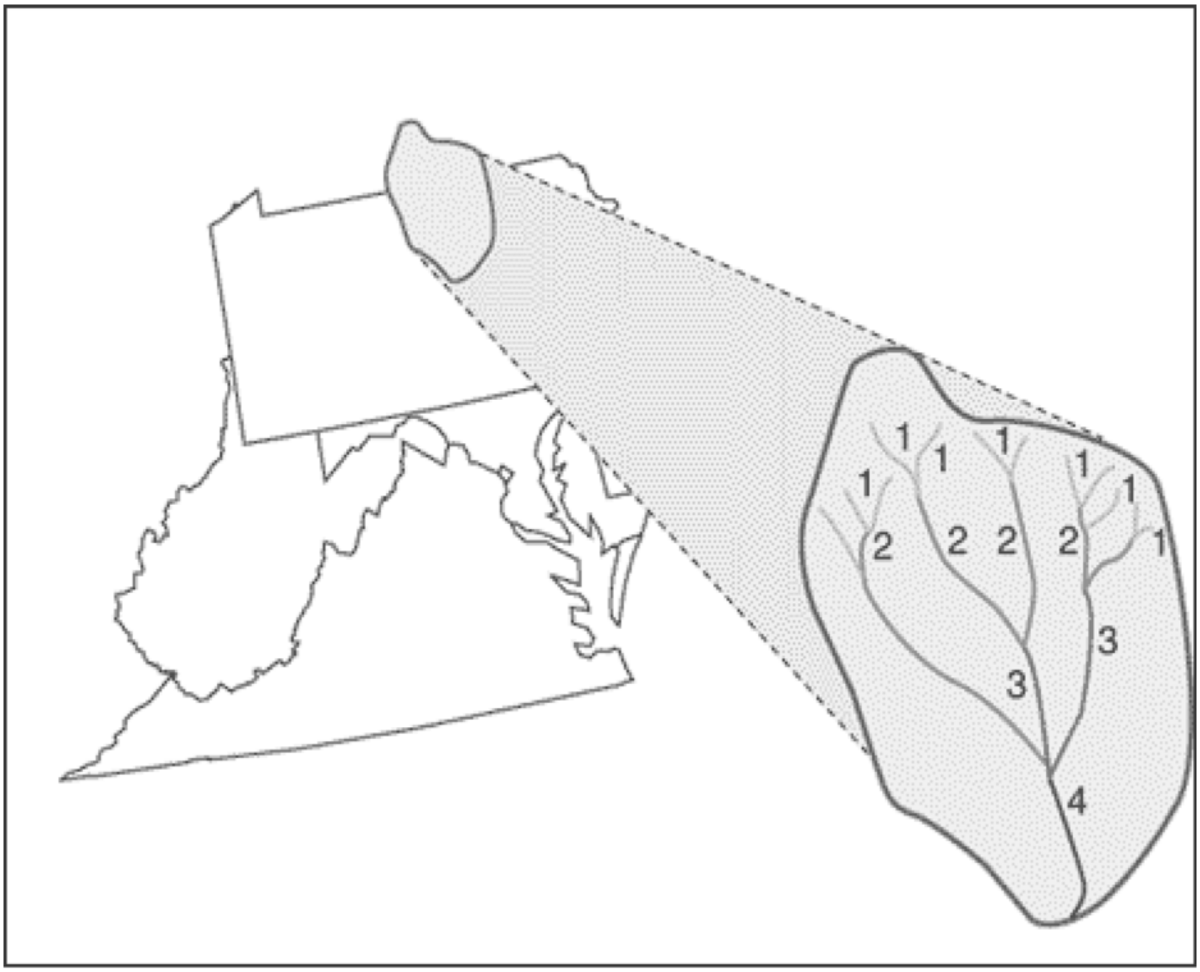


Figure 1. Identification of stream order for stream sections in a hypothetical watershed. Source: EPA. 2000. Mid-Atlantic Highlands Streams Assessment. EPA/903/R-00/015

Once you have located some possible streams to survey within the Refuge or Park using maps or photos, the next step will be to scout the length of the streams that fall within the Refuge or Park. To find a headwater area (spring, seep) occurring within the Refuge or Park boundaries (all upstream from the stream you were able to locate via maps and aerial photos), follow the main channel until it narrows to its origin. If the main channel is very large and/or fast-flowing, this may be a higher-order stream than you first thought (see Figure 1). Keep an eye out for smaller tributaries flowing into the main channel, and walk up one of these until you find its origin (spring or seep). You can turn rocks and logs along the stream as you go, recording what you find. This will serve as an inventory of the amphibians along the stream, and will also allow you to identify good stretches (rocky areas) where the transect-quadrat pairs can be conducted (see below for details about appropriate places for transect-quadrat placement).

In general, the first transect-quadrat pair should be conducted at or near the headwaters of a stream (the spring or seep source) or directly above a source of degradation, if possible (e.g., stormwater pipe). The other transect-quadrat pair should be conducted at a lower elevation, at least 50 m downstream, if feasible, or directly below the source of degradation (if this applies) from the first transect-quadrat pair.

The ideal situation would be to choose two streams that are not degraded and two streams that are degraded, conducting two sections (i.e., two transect-quadrat pairs) per stream. Alternatively, streams could represent both conditions if an undegraded stretch was surveyed directly above a source of degradation (e.g., cow pasture, point source) and a degraded stretch was surveyed below the source of degradation. However the stream sections are picked, we would like each Refuge or Park to survey at least 4 transect-quadrat pairs representing undegraded streams and 4 transect-quadrat pairs representing degraded streams. If there are no obvious sources of degradation in the Refuge or Park impacting streams, then streams representing different habitats (e.g., streams through woods versus grasslands), histories (e.g., burned, unburned), or covering the geographical extent within the Refuge or Park should be chosen.

Things to keep in mind when selecting stream stretches for transect-quadrat placement:

A) Salamanders can be found in very steep gradient streams. Steep channels featuring large boulders, waterfalls, and bedrock can provide prime habitat to stream plethodontids. However, such environments are difficult and dangerous to sample, so steep, boulder-strewn channels should be avoided. A suitable stream site will consist of riffle and run sections and pools (see Figure 2).

B) Cover is an important component of any animal's habitat. Salamanders are no different. Rocks, cobble, woody and other organic debris, and riparian vegetation all provide cover for salamanders. A barren stream bank will be devoid of plethodontid salamanders regardless of other habitat characteristics. Thus, focus on a stream reach that provides an abundance of cover. The ideal situation, from a stream sampling perspective, is a reach that contains a high proportion of cobble. In stream parlance, cobble refers to stones or "rocks" whose length ranges from 6.5–25.6 cm in length (2.6–10.1"). Although pebble (3.3–6.4 cm or 1.3 – 2.5") and boulders (> 25.6 cm or > 10.1") also provide cover, plots with a high proportion of cobble will give you more places to easily search. Sandy or bedrock-bottomed stream channels are often devoid of cobbles. The latter are also likely to be relatively steep. Avoid muddy streams, especially if cobble and other cover lie buried beneath the sand, silt, or muck. For example, small streams draining agricultural landscapes are prone to heavy sedimentation and fine sediments often cover the stream bottom; you may want to avoid these.

C) Stream salamanders require cool, moist environments. A broadly wooded stream reach is more likely to support a large community of stream salamanders than a stream reach lacking shade. Avoid open, sunny stream channels along pastures or agricultural fields. Plot sampling in these environments would also be very stressful to you -- consider spending over three hours in direct sun on a hot, humid day!

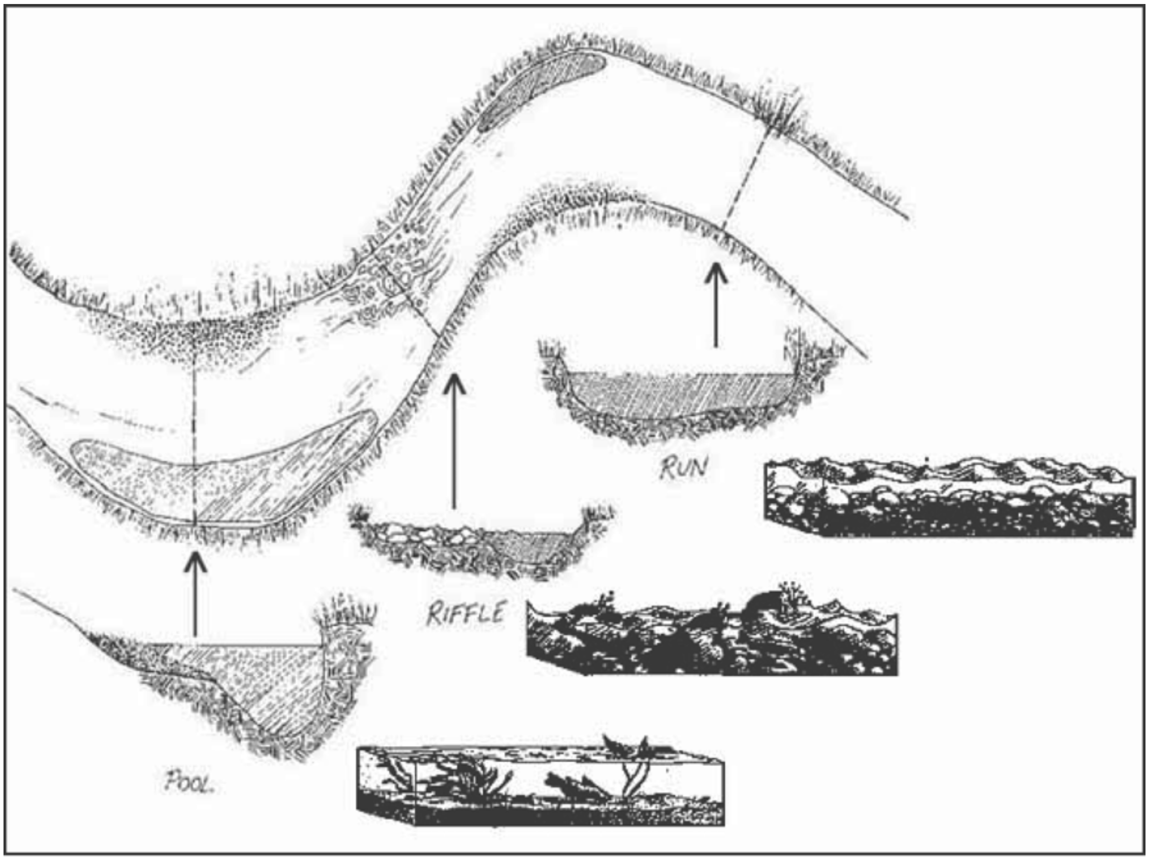


Figure 2. Location and cross section of riffle, run, and pool sections of a small stream. Source: EPA. 1997. Volunteer Stream Monitoring: a methods Manual. EPA 841-B-97-003

Transect Placement and Methods Summary

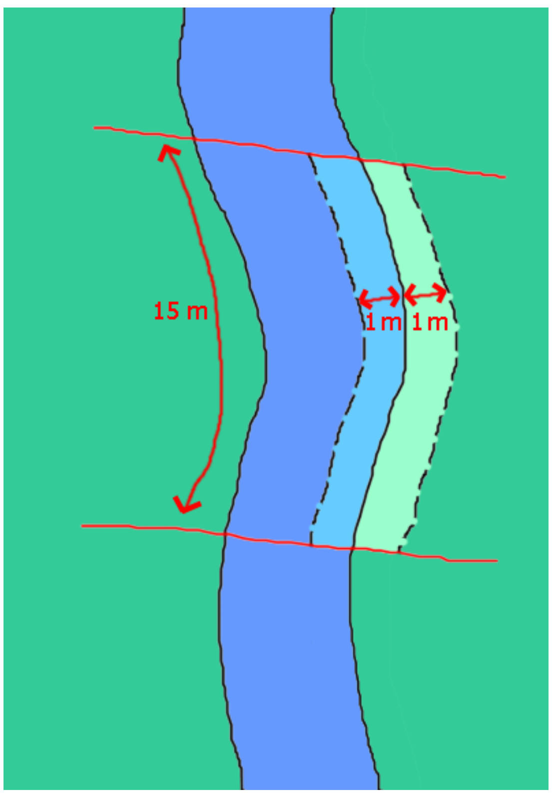
Transects (15 m x 2 m) should only be positioned in riffle and run sections of the stream (see Figure 2). Sampling in pools should be avoided. Pools tend to have steeper banks, especially beyond the water's edge, and are deeper. Sediments and fine particulate matter also collect in pools due to lower stream velocity. Visibility will be greatly diminished when this sediment in a pool area are disturbed. Although fine sediments also become trapped in riffle and run areas, the faster moving flow will remove suspended sediments quickly. Most importantly, however, sampling for aquatic salamanders will be easier in riffle and run areas than in pools. The former environments are also likely to support more animals. Where possible, position your transects along a straight portion of the stream and where the water's edge is straight and well defined. In some areas, the water's edge is broken by small pools and depressions. This makes the delineation of wet and dry areas more difficult.

The deeper the water, the more difficult it is to see or dipnet for aquatic larvae. Avoid positioning your transects in riffle and run areas deeper than 15 - 20 cm. Stream flow in riffle and run areas will be stronger than in pools. If not too strong, rapidly moving water can be used to your advantage, especially when raking through gravel to search for small larvae or to flush suspended sediments. On the other hand, very strong currents in deeper water should be avoided.

Remember, no cover, no animals. Look for areas with an abundance of terrestrial and aquatic cover, especially cobble. Areas that also contain ground vegetation and organic debris (e.g., logs, twigs, and dead leaves) are also favorable. Avoid areas that, despite having rock/cobble cover, appear scoured and are devoid of fine material. These areas probably experience periodic flushing and are unlikely to harbor many salamanders.

Once you have located your upstream-most transect, use flagging tape to mark the upstream-most point of the transect, labeling this with the stream name and "top transect 1". Follow the above guidelines to select the placement of your second, downstream transect, and flag the upstream-most point of this transect as well. Label this second, downstream transect with the stream name and "top transect 2". The transect you call "1" should always be upstream from the transect you call "2". The same is true for quadrats (see below).

Transects are 15 m long by 2m wide, and are conducted along either the right or left side of the stream, searching within 1 m from the water's edge along the bank and within 1 m from the water's edge in the stream channel (Figure 3a). If a stream is less than 1 m wide, survey 1 m on either side of the center of the stream (see Figure 3b). The first time you survey a transect, flip a coin to determine which side of the stream to survey. From now on, when you re-visit this stream, you will always survey on the side you first chose. Your downstream transect (transect 2) should be along the opposite side of the bank as your upstream transect (transect 1).



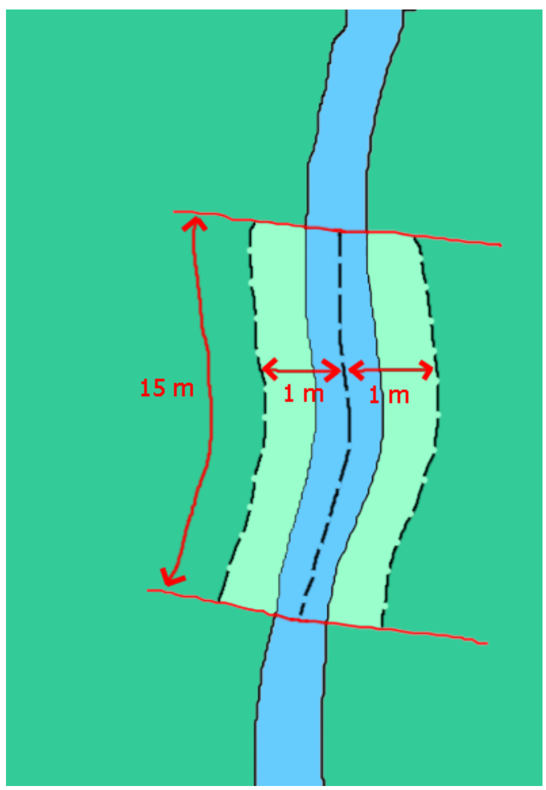


Figure 3a, b. Survey area for a stream greater than one meter wide (a, left diagram) versus a stream less than one meter wide (b, right diagram).

Surveyors will fill Unit Name, Stream Name, observer name, weather and other parameters on the datasheet first, but **leaving stream widths and depths to be measured at the end of the survey**. Then, they will mark out the transect with a meter tape, and begin the first pass at the downstream end of the transect, working upstream. Record begin time on the data sheet. If there are two people available, each can do a transect-quadrat pair (i.e., one person surveying transect 1 and quadrat 1, and the other surveying transect 2 and quadrat 2). Alternatively, one person can survey transect 1 while the other surveys quadrat 1; a similar arrangement can occur for transect 2-quadrat 2. **Do not put more than one person on a transect to 'split it up.'**

Surveyors will carefully turn over the surface layer of cover objects (i.e., rocks and logs) greater than 6.4 cm (2.5”) maximum width or length. Surveyors should turn over as many cover objects in the transect as possible, excluding those that are too heavy or too embedded to lift. As they go along, they will keep track of cover objects overturned using a tally counter. Any amphibians captured are placed in plastic zip-lock bags (see below for details on holding captured animals) and set aside **in the shade** until the pass is complete. Escapes should be recorded (see detailed methods below), but all attempts should be made to capture amphibians encountered. Escapes make population estimation more difficult because we can not use escaped salamanders in our tallies for removal passes

SURVEYORS MUST DO A **MINIMUM OF THREE PASSES** PER TRANSECT. From our recent analyses (soon to be published in a special volume of the journal Alytes), we have found that two passes per transect is just not enough to provide good population estimates, even if there are fewer captures on subsequent passes. The reason for this is mostly due to the already low sample sizes that we have been getting. In some cases you may have to do a FOURTH pass on your transect. Use the following as your decision rule:

> If, **per species of Eurycea or Desmognathus only**, your numbers are such that the totals for **(pass 1 + pass 2) ≥ pass 3**, then you can stop after the 3rd pass. If not, then you **must** do a fourth pass. You will never have to do a fifth pass. For example, in your first pass, you encounter 4 *Eurycea bislineata* (EBIS) and 2 *Desmognathus fuscus* (DFUS), in your second pass you encounter 4 EBIS and 1 DFUS, and in your third pass, you encounter 5 EBIS and 4 DFUS. For EBIS, plugging your numbers into the formula above gives us (4 + 4) > or = 5 which is true. However, for DFUS we have (2 + 1) > 4 which is false. Therefore, you must do a fourth pass due to the DFUS counts. (The formula should also apply to *D. monticola* and *D. ochrophaeus*).

Although you must also record and measure any *Pseudotriton* (PRUB, PMON) or *Gyrinophilus* (GPOR) species on your data sheet, disregard these species when tallying your counts to determine how many passes you need to do -- these species tend to be more rare and typically have lower detection probabilities, so this method may not be the best to estimate their populations.

At the end of each pass, record the total number of cover objects turned, and the end time for that pass. Then, process the captured amphibians in that pass, recording pass #, species, age class (larva, adult), snout-vent and total length and whether the amphibian is malformed or exhibits any signs of disease. Record any escapes encountered during the pass. This process is then repeated until the appropriate number of passes have been completed, at which time stream water depth and width at the beginning, middle, and end of each transect should be measured and recorded. After the final pass and all stream parameters have been taken, the animals can then be released. See below for detailed methods for transect sampling.

Quadrat Placement and Methods Summary

Place your quadrat just downstream of each transect in an appropriate riffle/run area, or if the stream is wide enough, you can place it on the opposite bank of transect, but on the downstream end.

Quadrats cover a surface area of 4 m^2 and measure 2 m x 2 m (approx. 6' 4" x 6' 4"). Sampling plots should include "dry" and "wet" portions of a stream. The dry and wet portions are the terrestrial and aquatic environments of the plot, respectively. Once you've identified a suitable bank area for a plot, place a flag at the edge of the water at the downstream-most end (Figure 4). Measure 2 m upstream along the water's edge and flag this distance. This will be the length of your plot. The land-water interface will be the midpoint of the plot, at least along its width. Next, measure 1 m (~ 3' 2") towards the terrestrial end at both the upstream and downstream flags. Flag both of these measurements. This area, which should measure approximately 2 m x 1 m, will be the dry portion of the plot. Do the same towards the aquatic end. This area, which should also measure 2 m x 1 m, will be the wet portion of the plot. When you've flagged all 4 corners, look at the plot. It should be square. If not, adjust if possible. While flagging, all disturbances to the plot interior should be minimized to the extent possible. Do not walk inside the plot unless you really have to and do NOT step on stones and rocks. Watch for salamanders attempting to escape.

Quadrats should be searched intensively, removing all cover objects as practicable. Quadrats represent destructive sampling, such that all rocks and gravel and debris within the quadrat are temporarily removed and only the underlying sand or bedrock is left. The goal is to ensure that no salamanders escape detection. Use amphibian catching techniques as described below. For quadrats, count and record the number of all surface rocks greater than 6.4 cm (2.5") maximum width or length overturned; do not count pebble or gravel. Once you have completely surveyed the quadrat and captured all salamanders within it, record and measure the salamanders (snout-vent length, total length). Return all the rocks to the quadrat, recreating the pre-sampling state of the quadrat as best as you can. Return the salamanders to the quadrat, allowing them to crawl back under the rocks in place.

IMPORTANT: To provide us with consistent data and detection estimates, please only have **one observer per transect and one per quadrat** (see below). We no longer can accept multiple observers per transect or quadrat. For example, if three people are available to survey your streams, one person should do each quadrat while the other two people do one transect each (i.e., one person on transect 1, another person on transect 2, and the third person surveying both quadrats one after the other). As another example, if you only have two people available, then each can do a transect-quadrat pair, where one person surveys the transect while the other surveys the paired quadrat, switching roles for the second transect-quadrat pair at the stream. Do not put more than one person on a transect to 'split it up.'

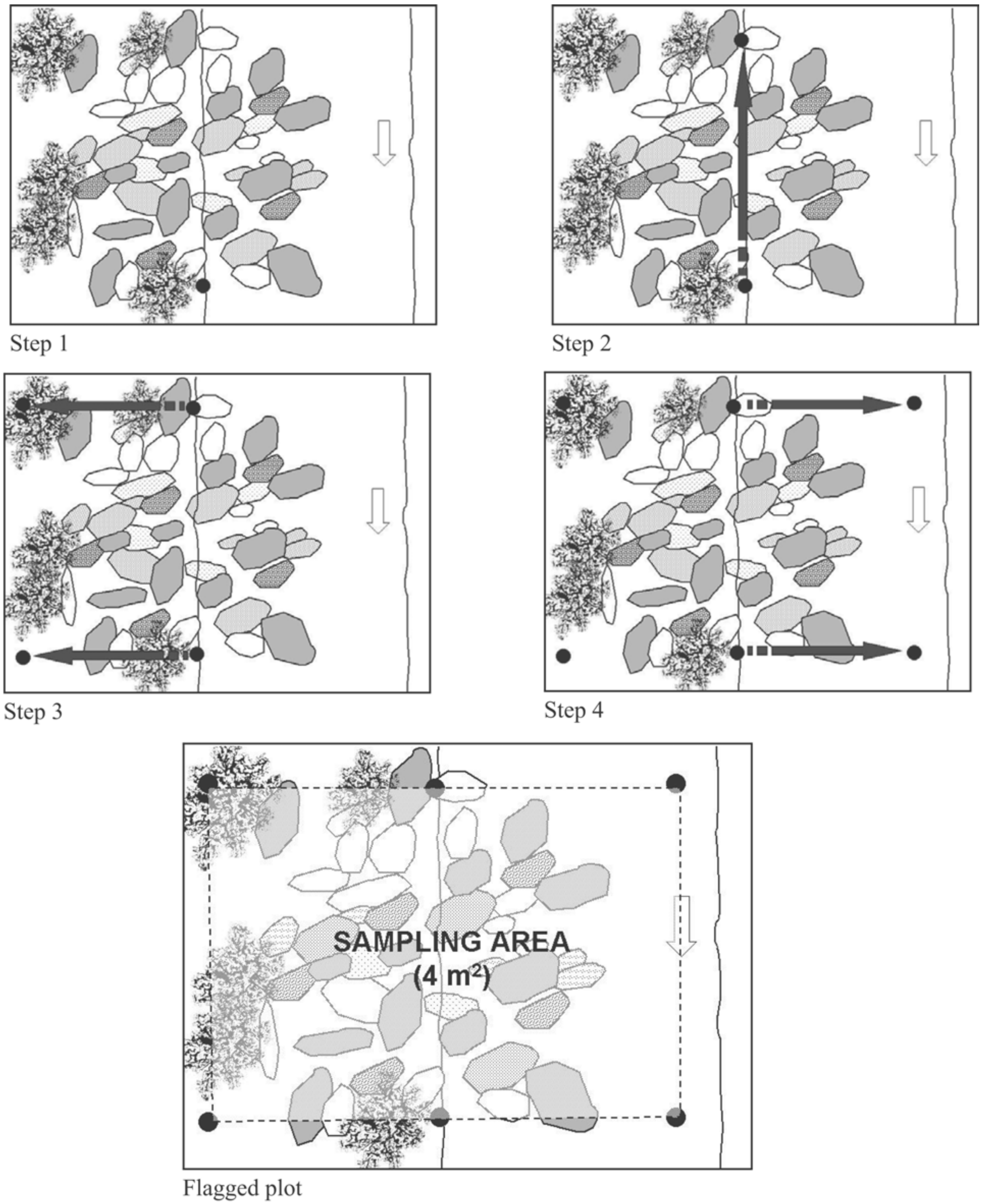


Figure 4 . Suggested sequence for flagging 4 m^2 quadrat.The two wavy lines represent the water's edge. Arrow identifies direction of stream flow. The quad measures 2 m x 2 m.

Detailed methods -- Transect:

1) On the Stream 15 x 2m Transect Data Sheet, record the Unit Name (name of Refuge or Park), Stream Name, Transect (circle whether you are surveying Transect 1 or 2), Date (e.g., 12 June), Air and Water Temperatures, Sky and Wind Codes, Turbidity (circle whether clear or turbid), Water Variables (please record pH, conductivity, and/or dissolved oxygen if you have meters available to measure these), and Date of Last Precipitation (e.g., 10 June).
2) Using a 50 m tape, mark out 15 m along the bank, following the curve of the wetted portion of the stream. Please be careful to avoid stepping on large rocks and logs within the transect, as salamanders may be under these.
3) Record habitat data for Transect 1 and 2 on the Stream Habitat Data Sheet before you begin each transect. See the Description of Data Fields section below for details.
4) Start at the DOWNSTREAM-MOST end of the transect. Use a meter stick to delineate how far you should search along the bank as well as in the stream channel. Just before you begin your survey, hold the meter stick so that one end is at the edge of the wetted portion of the stream, and the other is within the stream channel, perpendicular to the flow of the stream. Now you know how far into the stream you should survey to cover 1 m. Similarly, hold the meter stick with one end just at the edge of the bank and wetted stream area, and the other end on the bank. Now you know how far along the bank to survey to cover 1 m (see Figure 3).
5) Record the time that you begin your first pass on the data sheet under Pass 1 Begin Time using the 24-hr (military) format.
6) As you move upstream, use your tally counter to click the number of rocks and logs you turn over, and carefully turn over the surface layer of cover objects (rocks, logs) greater than 6.4 cm (2.5”) maximum width or length, turning over as many cover objects in the transect as possible, excluding those that are too heavy or embedded to easily lift. Do not 'dig out' embedded cover objects. Make sure to replace the cover objects that are lifted to their original position to minimize habitat disturbance.
7) In the stream channel, place the net firmly against the bottom substrate just downstream of your cover object. Next, lift the cover object in front of the net. The stream should be flowing in the direction of your net, such that larvae or adults are swept (or swim) right into your net after you lift the cover object. Try not to get too many rocks or pebbles inside of your net, as salamanders can be injured by these when you lift your net out of the water.
8) Check your net carefully for animals, but do not turn the net inside-out by lifting the bottom of the net above the rim of the net. Adult salamanders can jump right out of the net, and small larvae can quickly wriggle out and fall back into the stream. In your net, small larvae often look like worms with eyes until you get a closer look.
9) To capture larval salamanders, position the net or a zip-lock bag in the water in front of the salamander’s head and gently touch the tail; more often than not they will move forward into the net or zip-lock bag. Sometimes larvae are swimming around in the open and you can direct them into the net and then transfer them into the zip-lock bag from there. When you have a larva in your net and you are trying to transfer into your zip-lock bag, keep the animal in the net while filling your bag with 5-10 cm of water. Then hold the filled, open bag inside of your net and coax the larva into the bag. This way, if the larva misses the bag, it will still be in the net, rather than dropping back into the stream.

Do not attempt to grab the larva with your fingers – the gills are very fragile on these animals, and can easily be damaged. Instead, coax the animal onto part of the net where you can lift it (with your hand on the outside of the net) and drop it into the bag.

10) Try your best to minimize escapes. If you encounter a larva in the stream and it swims away before you are able to catch it in your net, remember this: larvae often appear to swim downstream, but usually they will dart back upstream. Sometimes they will 'flash' (a quick flick of the tail and a sideways turn) like a fish, but remain in place. The 'flash' will make you think they have darted away, but just wait for the sediment to clear and see if it is still there. If it is not there, slowly check adjacent rocks, and be sure to turn a few rocks upstream as well.
11) When turning cover to find an elusive animal, remain calm and turn cover SLOWLY, as the animal may be staying still and hoping you don't notice it. If you flip cover too quickly with an escaped animal, it may be scared further and further away. For adults, try your best to watch in which direction the salamander has gone, and again, SLOWLY turn cover objects trying to find it. Always make sure your net is ready.
12) On the banks, have your net ready to catch adult amphibians from under overturned rocks and logs. For adults, use the same tactic as for the larvae (see above), or you may have to go after them using your dip net or hand if they try to escape. Stream salamanders can be quite fast! Once you have turned over the rock or log, you might see movement right away, in which case you need to catch it quickly! Once you have the salamander in your hand or net, transfer the salamander to a zip-lock bag as described above for larvae. If a salamander escapes, write down the information about the species (put a question mark by the species if you are uncertain) and estimate the total length. In this case, make sure to write "ESCAPE" in the Notes section of the data sheet.
13) After the first pass, measure all the amphibians caught (snout-vent length, total length) and keep them in the shade in their plastic bags. Make sure to place the bags at the edge of the stream in a pool in the shade (make sure they don't float away!) to keep them cool. Do not return these salamanders yet to the stream. Once they are measured, you have the option to transfer these first pass amphibians to larger same age-same species containers (e.g., plastic tubs with water for larvae, spackle buckets with lids and a little water for adults) so that they have more room to move around. However, do not mix species and age classes (particularly since larger adult and larval salamanders such as northern spring salamanders, red and mud salamanders will eat smaller salamanders, such as northern two-lined salamanders and young dusky salamanders and their larvae). Again always keep the animals cool and do not return these salamanders to the stream until after your final pass.
14) Next, conduct the second pass, turning over approximately the same number of rocks or logs as you did during the first pass to keep the effort consistent between passes. Follow the same rules as above, measuring and recording the salamanders from your second pass. Put these salamanders in the shade. Next, conduct your third pass as above and measure and record the salamanders from your third pass onto the data sheet.
15) Follow the decision rule above and determine if you need to do a fourth pass; if so conduct it as above, recording begin time, end time, number of rocks or logs turned, and measuring any animals found.

16) After your final pass, measure stream channel wetted widths and channel depths at the beginning, middle, and end of the transect (see “Description of Data Fields” below for more details).

Detailed methods -- Quadrat:

1) On the Stream 4 m^2 Quadrat Data Sheet, record the Unit Name, Stream Name, Date (e.g., 12 June), Observer Name(s) for Quadrat 1 and 2, Distance from the beginning of the paired Transect, and the Begin Time when you start sampling. Sampling should proceed in a systematic fashion. Begin from the downstream end and slowly work your way upstream through the quadrat.
2) Follow the procedures as described above (Detailed methods – Transect), with the exception that cover objects are not returned to their original positions but instead are removed from the quadrat. Cover objects are not returned to their original positions until the quadrat is completed.
3) Place all cover items outside the quadrat. When you are done, there should be no cover items inside (Figure 5). Sampling in the aquatic portion should proceed in the same manner. You’ll find that placing cobbles and rocks along the outer perimeter of the quadrat, beginning with the downstream end first nearest to the dry bank, will slow down the current and let you see the bottom easier. Always position your dipnet on the downstream side of all submerged cover before lifting items out of the water. More often than not and depending on the amount of stream current, hidden larvae will end up in the net when you remove the item.
4) Once you’ve removed the larger rocks and cobble, try also raking the substrate with your fingers while trailing the dipnet behind and on the downstream side of your hand. After a few rakes, sort through the material trapped in the net. Inspect diligently for larvae before disposing of the contents in your net. Both methods do not really require you to see salamanders before catching them. However, you should always be on the lookout. Larval northern red and northern spring salamander have salmon-colored bodies and despite their larger size can easily be overlooked if the stream bottom is similarly colored.

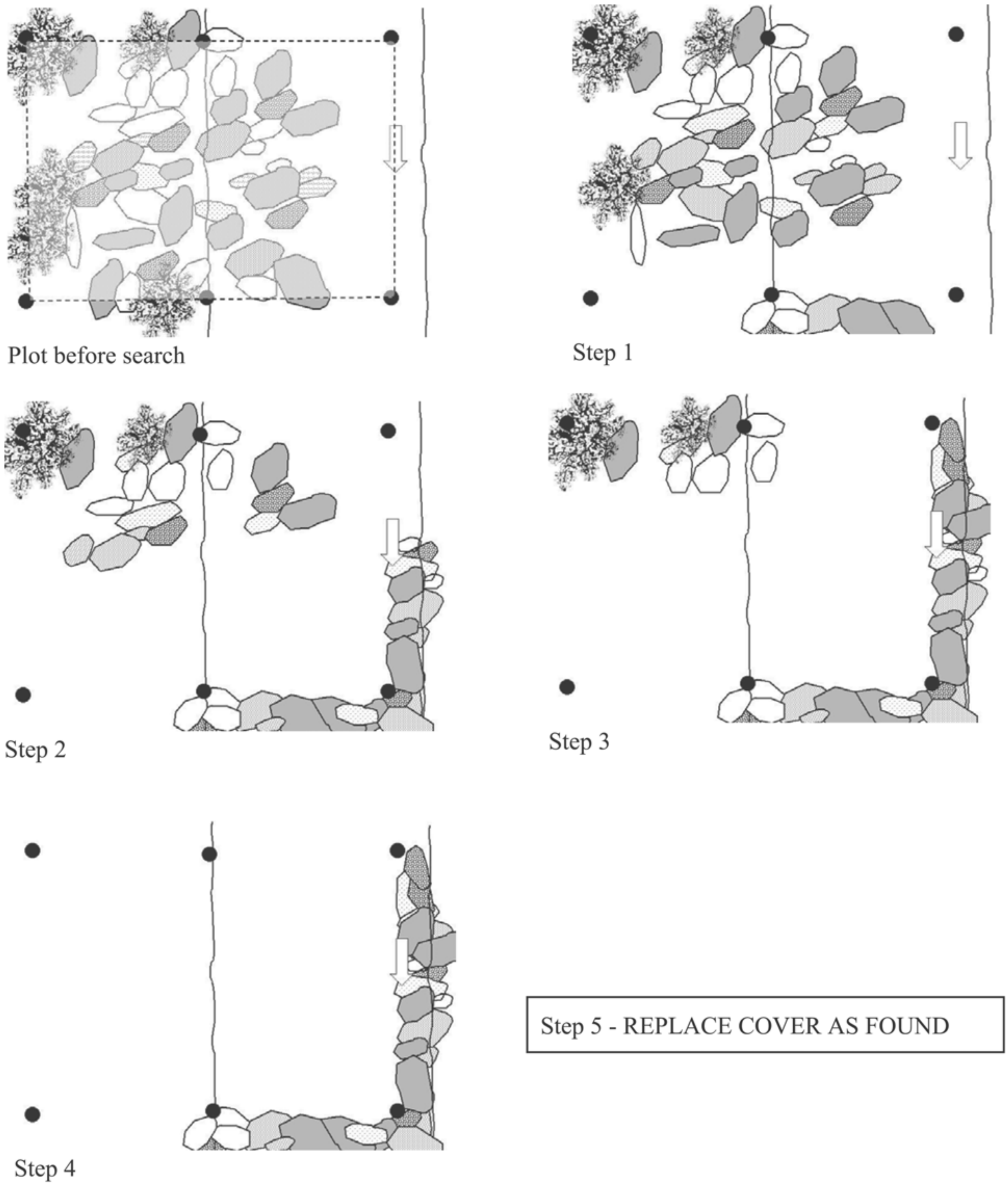


Figure 5. Suggested sequence to systematically sample 4 m^2 plot. Removal of cover begins on the downstream end of plot and proceeds upstream. Wet and dry bank areas in plot are sampled simultaneously by 2 or more individuals. Positioning of removed cover along outer edge of wetted portion of plot improves visibility.

Amphibian Capturing, Handling, and Photodocumentation of Species and Streams

All captured amphibians (larvae and adults) should be placed into sealable zip-lock plastic bags for identification and measuring. Larvae (with gills) must be kept in plenty of stream water such that their entire body is covered with water. Adults (without gills) should have access to a little water (enough to keep moist but not to drown). Adult plethodontid salamanders are lungless and respire through their skin. Make sure the zip-lock bag has air for adults so they are not stuck between sheets of plastic in the zip-lock bag. Amphibians should be kept in the shade at all times to avoid overheating. At the end of sampling (i.e., after the quadrat or final pass of the transect), all amphibians must be returned to the quadrat or transect. To return salamanders to the exact point of capture, you can use numbered wire flags to mark the capture point, writing the same number of the wire flag onto the zip lock bag in which the amphibian is placed. Otherwise, release the salamanders as close to their point of capture as possible. Pour larvae and let adults crawl out of the zip lock bags next to a rock that they can swim or crawl underneath. It is highly recommended that you take a camera with you into the field (digital cameras preferred) so that you can take representative pictures of the stream sections and species and age classes you encounter, malformed amphibians, or any other unusual sightings.

Dipnets

Dipnets are indispensable for capturing aquatic larvae, and will also greatly facilitate the capture of adults and minimize their injury. We recommend the larger-sized rectangular green aquarium dipnets with fine mesh, typically sold as aquarium nets in pet stores.

Field Work Code of Practice

Biologists can spread various diseases among sites that can impact amphibians (e.g., chytrid fungus, iridovirus, ichthyophonus fungus). Transfer of disease agents among sites can be avoided by: 1) designating specific dip nets for exclusive use at each stream (tie flagging tape to each dip net and write the stream name on the tape), and 2) cleaning and bleaching boots or other equipment thoroughly between sites. Below are highly recommended procedures for boot cleaning between streams during all amphibian survey work:

1) Take a stiff scrub brush, a spackle bucket half-filled with water (covered by a lid), and a 50% solution of bleach:water in a squirt bottle with you into the field.
2) Clean boots of all wet or dried mud using a stiff scrub brush and the bucket of water.
3) After boots are cleaned of mud, spray the boots with a 50% solution of bleach:water.
4) Rinse the boots by dipping them into the spackle bucket with water.

DESCRIPTION OF DATA FIELDS

Unit Name: Record the name of the Refuge or Park (e.g., Canaan Valley NWR) and Subunit if applicable (e.g., Great Meadows NWR-Oxbow subunit).
Stream Name: Record the name of the stream (e.g., Cow Knob Creek).
Date: Record Day and Month (e.g., 20 June). The Year (2004) is already on the data sheet.
Observer Name(s): Record the name of the person conducting the amphibian survey.

Recorder Name: Record the name of the person recording the data on the data sheet.
Transect: Circle whether it is transect 1 (upper, headwater) or 2 (lower) for that stream.
Distance from Transect to Quadrat (m): Record the minimum distance in meters from the transect to the quadrat.
Date of Last Precipitation: Record (as best you can remember) the date when it last rained.
Air Temperature (ºC): Record 1 meter above the ground in the shade.
Water Temperature (ºC): Record about 1/3 meter out from shore 2 cm below water surface.
Other Water Quality Variables (Optional): Water pH, Conductivity, Dissolved Oxygen.
Turbidity: Record whether water is clear or turbid (e.g., cloudy with algae, muck or particulates).
Sky Code: Use the following codes:

0 = Clear or few clouds (< 20% of sky covered with clouds)
1 = Partly cloudy or variable (20-50% of sky covered with clouds)
2 = Cloudy or overcast (> 50% of sky covered with clouds)
3 = Fog
4 = Mist or drizzle
5 = Showers or light rain
6 = Heavy rain (don't do survey!)
7 = Sleet or hail (don't do survey!)
8 = Snow (don't do survey!)

Wind Code: Use the Beaufort wind scale codes

0 = < 1 mph, calm, smoke rises vertically
1 = 2-3 mph, light air movement, smoke drifts
2 = 4-7 mph, light breeze, wind felt on face, leaves rustle
3 = 8-12 mph, gentle breeze, leaves in constant motion, raises dust
4 = 13-18 mph, moderate breeze, small branches move
5 = 19-24 mph, fresh breeze, small trees begin to sway
6 = 25-31 mph, strong breeze, large branches move (go home!)
7 = 32-38 mph, near gale, large trees begin to sway, difficult to walk (go home!)

Stream Wetted Width (cm): Record stream wetted width (from one edge of the existing water to the other, NOT the distance from bank or channel edge to bank or channel edge) at the beginning, middle and end of the transect.
Maximum Channel Depth (cm): At the same places you record stream width, record maximum channel depth along your stream width measurement.
Begin Time: Record the hour and minute survey begins (use 24 hour clock) for each quadrat and transect pass (e.g., 1310).
End Time: Record the hour and minute survey ends (use 24 hour clock) for each quadrat and transect pass.
Cover Objects: Record the number of overturned rocks and logs for each pass (transect) or quadrat.
Species: Record the species observed writing the full common or scientific name or using the following code (e.g., DFUS = *Desmognathus fuscus*, EBIS = *Eurycea bislineata*, GPOR = *Gyrinophilus porphyriticus*, PRUB = *Pseudotriton ruber*).
Age Class: Record whether the species is a larva (gills present) or an adult (no gills present).
Snout-Vent Length (mm): Measure the snout-vent length (snout to posterior end of the cloaca). For very small larvae, sometimes the cloaca is not very visible. In these cases, just measure to behind the hind limbs.

Total length (mm): Measure the total length (snout to end of tail). In Notes, record if tail cut or missing.
Notes: Record whether amphibian is an escape, whether it is nesting (provide details about the number of eggs) or whether there is anything else unusual about it (e.g., malformed – describe malformation in detail, missing tail, etc.).
Fish? Record whether present (Yes) or absent (No). Describe in Notes section.
Crayfish? Record whether present (Yes) or absent (No).
Aquatic Invertebrates? Record whether present (Yes) or absent (No). Describe in Notes section.

Data Fields only on the Stream Habitat Data Sheet

--IMPORTANT: As with our vernal pool sampling, **UTMs can not be accepted for stream locations**. Please record stream coordinates in degrees, minutes, decimal seconds (ddd mm ss.ssss) using North American Datum 1983 (NAD 83) with Spheroid GRS 1980 (GRS 80). Also record the estimated position error (often seen in your GPS display as EPE) in +/- X meters. Record these lat/longs from the top (most upstream point) of BOTH transect 1 and transect 2 (i.e., 2 GPS coordinates per stream).
Latitude: Record the upstream coordinate for each transect (ddd mm ss.ssss).
Longitude: Record the upstream coordinate for each transect (ddd mm ss.ssss).
Stream Channel Modification: Select one of the following:
- 1 = Stream channel is natural without modification
- 2 = Stream channel is modified (e.g., with cement, pipe, dredged, etc.); describe

Stream Order: Select the best description of the stream order category:
- 1 = Starting from the headwaters; headwater area
- 2 = Stream segment from unbranched tributary further downstream from headwater area; first order
- 3 = Stream segment resulting from the joining of 2 or more unbranched tributaries; second order

Slope: Record the slope of each transect from highest to lowest point (between 0º and 90º).
Flow Regime: Record % of each transect that is covered by each of the categories below:
- % Dry: No visible moisture or water
- % Moist: No flow, but moist soil
- % Seep: Slow flow, trickle or drip
- % Pool: Standing/stagnant water
- % Riffle: Riffles/small waves, not caused by obstruction
- % Run: Swiftly-moving, smooth surface current

% Substrate Embeddedness: Record the percent (0-100%) of visible vertical surfaces (rock) that are surrounded by line sediment or flocculent material (buried or embedded in either silt, fine sediments or sand) (Lowe and Bolger, 2002).
% Substrate Type: Record the % substrate covering each transect; must sum to 100%.
- % Sand (< 2 mm, gritty texture)
- % Gravel (2-32 mm)
- % Pebble (33-64 mm)
- % Cobble (65-256 mm)
- % Boulder/Boulder slabs (> 256 mm)
- % Bedrock

% Silt (particles < 2 mm, greasy texture when rubbed with fingers; clay and fine organic)
% Detritus (partially or undecayed sticks, wood, leaves or other plant material)
% Clay/Hardpan (hard and gummy clay, hard to penetrate)
% Muck (decayed organic matter with little or no clay content)
% Artificial (cement, pipe, etc.)

Riparian Width: Check the appropriate width of the adjacent forested buffer area along both the right and left banks of the stream.

Land Use: Record % land use adjacent to the site (within 50 m on both sides); must sum to 100%.
% Agriculture/Field
% Pasture
% Industrial/Urban
% Suburban/Residential/Park
% Mining/Construction
% Mature Forest
% Immature Forest/Shrub
% Meadow/Marsh
% Road
% Other: Describe

Disturbance or Habitat Type Represented: Describe

EQUIPMENT LIST

Aquarium Dip Nets (fine mesh – 6” x 8” or 8” x 10”)
Tally Counters (clicker counters)
Plastic Tubs with Lids and/or Spackle Buckets
Rubber Boots (knee-high) or Hip Waders (for deeper streams)
Meter Sticks
Water Quality Equipment (if available)
Digital Camera (with film and card)
GPS Unit
Zip-Lock Bags (Sandwich size and larger with triple color) – Box of 100
Rulers (30 cm)
50 m fiberglass tapes
Data Sheets (preferably on Rite-in-the-Rain paper)
Clipboard
Pencils & Sharpies
Wire Flags
Flagging Tape
Bug Repellent (if necessary, though do not apply to hands or other parts of body that may come in contact with amphibians or the stream itself)
Bleach solution in Spray bottle
Scrub brush

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